S/N 10/626502 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	SUMIDA ET AL.	Examiner:	B.J.FETTEROLF
Serial No.:	10/626502	Group Art Unit:	1642
Filed:	JULY 23,2003	Docket No.:	14633.0001US01
Title :	AGGLUTINATION ACCELERATOR FOR IMMUNOLOGICAL MEASUREMENT		

CERTIFICATE UNDER 37 CFR 1.6(d):
I hereby certify that this paper is being transmitted by facsimile to the U.S. Patent and Trademark Office on December 27, 2006.
By: Lisa Dorn
Name LISA DORN

Mail Stop:AF
Commissioner for Patents
P.O.Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Kyoichi Sumida, hereby declare as follows.

I graduated from Hiroshima University in March, 1995.

I began employment with Wako Pure Chemical Industries, LTd., the Assignee of the above-identified application in April, 1995 and have been engaged in said company, since that time, in the study of immunological reagent and assay thereof in Osaka Research Laboratories.

I am co-inventor of the above-identified the application and am well aware of the September 27, 2006 Office Action.

The following experiments were conducted by me.

Experimental data -Measurement of blank values using various polymers as an agglutination accelerator-

(1) Preparation of an anti-human PSA antibody sensitized latex test solution

A mixture of 0.5 ml of 50 mM borate buffer (pH 7.1) containing 0.7 mg of anti-human PSA mouse monoclonal antibody (made by Wako Pure Chemical Industries, Ltd.) and 0.5 ml of 50 mM borate buffer (pH 7.1) containing a polystyrene latex (particle size 0.28 μm, made by Sekisui Chem. Co. Ltd.) suspended in 2% by W/V, was reacted at 25 °C for 2 hours. Subsequently, the latex separated by centrifugation was washed with 50 mM borate buffer (pH 7.1), and said latex was suspended in 50 mM borate buffer (pH 7.3) containing 0.5% by W/V of BSA so that the concentration of the latex became 0.1% by W/V, and the thus obtained mixture was used as an anti-human PSA antibody sensitized(immobilized) latex test solution.

(2) Sample

A solution of 10 mM phosphate buffer (0.85% NaCl) containing 1.0% by W/V of BSA was used as a sample.

(3) Reagents

i) Test solution No. 1

A solution of 100 mM HEPES-NaOH buffer (pH 7.0) containing 1% of one of the following polymer as an agglutination accelerator, 0.5% of BSA and 1% of NaCl was used as test solution No. 1.

Polymer1: Copolymer of 2-methacryloyloxyethylphosphorylcholine / n-butyl methacrylate (the ratio of monomer = 8 / 2)

Polymer5: Copolymer of 2-methacryloyloxyethylphosphorylcholine / benzyl methacrylate (the ratio of monomer = 8 / 2)

Polymer6: Homopolymer of 2-methacryloyloxyethylphosphorylcholine

ii) Test solution No. 2

The anti-human PSA antibody sensitized (immobilized) latex test solution prepared in (1) was used as a test solution No. 2.

(4) Assay method

Assay was performed under the following measuring conditions by using a BM-8 automatic analyzer made by JEOL Ltd. The assay was repeated 5 times.

Sample: 10 μl
Test solution No. 1: 90 μl
Test solution No. 2: 30 μl
Assay method: 2-point end method (34 point and 65 point)
Main wavelength: 694 nm

(5)Results

(ng/ml)	Polymer5	Polymer1	Polymer6
1st	4	5	12
2nd	3	8	-4
3rd	-4	-8	-11
4th	-3	5	6
5th	2	-5	-5
Variation	8	13	23

As is clear from the results, blank value obtained by using the Polymer1 shows a level of variation about 1.5 times that obtained by using the Polymer5. Blank value obtained by using the Polymer6 shows a level of variation about 8 times that obtained by using the Polymer5. Thus, it is found that Polymer5 is a superior agglutination accelerator which has little influence on blank value.

I, the undersigned declarant, declare further under the penalty of perjury of the laws of the United States that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Signed this December 27, 2006

Kyoichi Sumida

Kyoichi Sumida

Signature

Review article *Int J Urol 1994;1:99–113*

PROSTATE-SPECIFIC ANTIGEN AS A TUMOR MARKER IN PROSTATE CANCER

*Manabu Kuriyama**
Department of Urology, Gifu University School of Medicine, Gifu, Japan

Key words: prostate-specific antigen, tumor marker, prostate cancer, tissue-specific antigens, early detection, monitoring

INTRODUCTION

Prostate cancer has become one of the leading malignant diseases in males in the United States,[1-3] being the most frequent in terms of incidence and second in terms of mortality. Even in Japan, the number of patients with prostate cancer is increasing and more than 2 in 100,000 habitants die annually from it. The cancer displays some special characteristics such as a racial difference in the incidence of the clinical cancer,[3] a slow development in some cases, good response to anti-androgen therapy in about 80% of cases and a lack of suitable therapy after relapse.[4-5]

Numerous efforts have been made to reduce the mortality due to this cancer.[4,6-11] To this end, the early detection of prostate cancer, and adequate monitoring during treatment and detection of relapse or recurrence have been thought to be very important. At the present time, early detection of prostate cancer is not necessarily associated with a good prognosis[12-15] although the distribution of stages, which is believed to be the major risk factor of prostate cancer, differs between those cases found by mass screening and symptomatic ones.[16]

To diagnose and monitor prostate cancer, acid phosphatase [orthophosphoric monoester phosphohydrolase, EC 3.1.3.2., (AP)]as well as digital rectal examination (DRE) are routinely used. The significance of AP as a tumor marker of prostate cancer was first described by Gutman et al. in 1936,[17] who found a high correlation between serum AP activity and the occurrence of prostate cancer, especially in the metastasized stages to bones. As AP is widely distributed in red blood cells, leukocytes, platelets, and organs such as liver, spleen and kidneys as well as prostate, diseases other than prostate cancer may also cause elevated serum AP activity. Therefore, low specificity is the major problem associated with the use of AP as a tumor marker. In 1957, Schulman et al.[18] purified a specific fraction of AP from prostate tissues (prostatic AP, PAP) and performed a clinical evaluation using single radial immunodiffusion. Following this, several immunoassays for the detection of PAP were developed by Cooper and Foti,[19] Vihko et al.,[20] Chu et al.,[21] Choe et al.,[22] and Lee et al.[23] Today, PAP is usually assayed by radioimmunoassay (RIA)[24] or enzyme immunoassay (EIA).[25] The clinical evaluation of PAP assays has resulted in a highly specific and low sensitive technique, especially in the early stages of prostate cancer.[26-28] Therefore, when the clinician finds a high serum PAP in a patient, it strongly suggests advanced prostate cancer. However, if a patient has a normal serum PAP level, prostate cancer cannot be excluded.[27]

Another tumor marker of prostate cancer, distinct from PAP, has also been studied for a long time. Prostate-specific antigen (PSA) was identified and purified to homogeneity from human prostate by Wang et al. in 1979.[29] Since then, PSA has been studied by many investigators all over the world and its superiority as a tumor marker of prostate cancer has been demonstrated. So much so that it is now one of the best tumor markers available.

In this review, we describe the identification, biochemical characterization and clinical significance of PSA. Recent advances involving PSA density and the PSA-protease inhibitor complex are also discussed. Finally, the standardization of PSA assays is examined. Many excellent reviews of this field have been published mainly in the United States and Europe,[30-42] and here the achievements of Japanese researchers are highlighted.

IDENTIFICATION, PURIFICATION, AND BIOCHEMICAL PROPERTIES OF PSA

Since 1960, many efforts to find tumor markers of prostate cancer other than PAP have been made by Flocks et al.,[43] Ablin et al.,[44] Li et al.[45] and Sensabaugh.[46] Wang et al. of Roswell Park Memorial Institute, USA, (RPMI) were the first to identify and purify to homogeneity a glycoprotein immunologically distinct from PAP and named it prostate-specific antigen (PA); in 1979 the name was changed to PSA.[29] In their paper, they reported that the concentration of PSA in benign prostatic hyperplasia

*Requests for reprints: Department of Urology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500, Japan.

0919-8172/94/0102-0099/US$03.00

(BPH) tissue was 95 μg/g and 700 μg/ml in seminal plasma. Both purified PSAs were found to be immunologically identical and have similar biochemical properties.[47] Following that, Papsidero et al.[48] of the same department identified PSA in human serum and confirmed that this material was identical to that purified directly from prostatic tissue. This suggested that PSA could be used as a sero-marker of prostate cancer.

PSA is a glycoprotein containing about 10% carbohydrate and its molecular weight is 33,000, determined by Sephadex G-75 (Pharmacia Biotech AB, Uppsala, Sweden) column chromatography. Upon SDS-polyacrylamide gel electrophoresis, purified PSA gives a single band with a location corresponding to a molecular weight of 34,000, indicating that it contains a single polypeptide. Sucrose density gradient ultracentrifugation showed a single protein band with a sedimentation coefficient of 3.1 S. The isoelectric point of the purified preparation was found to be approximately 6.9.[26,47] PSA has 240-amino acid residues and 4 carbohydrate side-chains. The N-terminal amino acid is isoleucine and the C-terminal residue is proline. The N-linked carbohydrate side-chain is at amino acid 45; aspargine and the O-linked carbohydrate side-chains are at amino acid 69, serine, at 70, threonine and at 71, serine.[49] The complete gene encoding PSA has been sequenced and localized to chromosome 19. The gene was found to be approximately 6 kb in size and composed of 4 introns and 5 exons.[50] PSA and the proteases of the kallikrein family of genes were found to exhibit a high degree of homology.[51] PSA is a kallikrein-like serine protease that is produced exclusively by the epithelial cells lining the acini and ducts of the prostatic gland. PSA is secreted into the lumina of the prostatic ducts and is present in seminal plasma. In seminal fluid, PSA is involved directly in the liquefaction of the seminal coagulum that is formed at ejaculation.[52] As far as specific enzymatic activity is concerned, Ban found it to possess chymotrypsin (ACT)- and trypsin-like activity.[53] These observations led to the recent use of serum PSA-ACT or free PSA determinations for the differential diagnosis of prostate cancer from BPH, to be discussed later. The protein, γ-seminoprotein (γ-Sm), which was the first prostate-specific antigen to be studied, was purified by Hara et al.[54] and has been mainly evaluated in Japan.[55-58] This and p30 identified by Sensabaugh are thought to be identical immunologically and functionally to PSA.[26,59-60] Seminal vesicle-specific antigen (SVSA) is also thought to be the substrate for PSA during semen liquefaction.[50] The characteristics of PSA are summarized in Table 1 and compared with those of PAP and γ-Sm.

CLINICAL CHARACTERISTICS OF THE PSA ASSAY

Serum PSA was first measured by researchers at the RPMI. Papsidero et al.[48] were able to measure PSA in 17 of 219 patients with advanced prostate cancer using rocket immunoelectrophoresis. Then Kuriyama et al.[61] developed a highly sensitive enzyme immunoassay (EIA, sensitivity; 0.10 ng of PSA/ml serum) and studied PSA levels using serum samples collected during the National Prostatic Cancer Project in the USA. They found that the mean serum PSA of 51 normal men was 0.47 ± 0.66 ng/ml. When a value of 1.8 ng/ml (mean + 2SD) was taken as the upper limit of normal, it was found that 68% of BPH cases had an elevated PSA. So too did 63% of stage A prostate cancers, 79% of stage B, 77% of stage C and 86% of stage D. This report is the first detailed clinical evaluation of PSA.

Table 1. Physico chemical characteristics of PSA compared with γ-Sm and PAP.

	PSA	γ-Sm	PAP
Characteristics	Glycoprotein	←	←
Molecular weight	33,000–34,000	23,000, 33,000	100,000
Subunit	Monomer	←	Dimer
Isoelectric point	6.9	5.8–7.1	4.2–5.7
Sedimentation coefficient	3.1 S	2.55 S	5.7 S
Carbohydrate content	10%	12%	7%
Ammonium sulfate precipitation	45–50%	←	50–70%
Amino acid Number	237, 240	237	784
N-terminal	Asparate	Asparate	Arginine
Optimum pH	7.0	←	4.8–6.0
Cell localization in prostate	Epithelial cell	←	←
Secretory nature	Present	←	←
Biological half-life	2.4 d		1.2 h
Function	Serine protease	←	Phosphatase

As Osterling mentioned in his review,[40] it is important for the reader to remember which assay system is used to determine PSA when its various clinical characteristics are discussed. The first available commercially PSA assay kit was distributed by Hybritec, San Diego, USA in 1984[62] (Tandem-R PSA), then Yang et al. developed another radioimmunoassay (RIA) in 1985 (Pros-Check PSA; Yang Laboratory, Bellevue, USA).[63] Both kits are made in the USA and have been widely used there and in Europe. In Japan, Miki et al.[64] evaluated Eiken PSA (RIA) (Eiken Chemical Co., Ltd., Tokyo, Japan) in 1984 and Kuriyama et al.[65] assessed an EIA (Markit-F PA; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) for the determination of PSA in 1987. Japanese urologists, therefore, are used to Eiken PSA or Markit-F PA assays. Some differences in the clinical data have been found when PSA evaluations have been carried out. Recently, more than 10 different assay kits have become available in Japan which has caused some confusion in evaluating the clinical data.

Stamey et al.[34] and Oesterling et al.[66] simultaneously and independently calculated the half-life of serum PSA. Using Pros-Check PSA, Stamey et al. determined the half-life to be 2.2 ± 0.8 days, whereas Osterling et al. obtained a slightly longer half-life of 3.2 ± 0.1 days using the Tandem-R PSA assay. Compared with PAP, and irrespective of its relatively small molecular weight, PSA has a long half-life. Both groups found it necessary to wait for 2–3 weeks to obtain a baseline level of serum PSA after prostatic manipulation such as occurs during transrectal ultrasound, biopsy, and various treatments involving the prostate.

Serum PSA concentrations do not appear to fluctuate unpredictably or display a circadian rhythm during a 24-hour period, compared with PAP, following the observations of Dejter et al.[67] and Maatman.[68] Since serum PSA values changes very little during any 24-hour period, there does not appear to be an optimum time for its measurement. Stamey et al.[34] found that serum PSA decreases 18% on average, 24 h after hospitalization, but the exact reason for this is not yet understood.

The effect of DRE has been studied as one of a number of factors affecting serum PSA determination.[69–70] Neither PSA nor PAP levels changed significantly 5 to 39 min after DRE, as shown by Brawer et al.[70] In contrast, Stamey noted a 1.92-fold increase in post-DRE serum PSA levels.[71] He also found a 4-fold increase due to cystoscopy and a 57-fold elevation after needle core biopsy of the prostate.[71] Transrectal ultrasonography alone, however, appeared to have very little effect on serum PSA.[72] Falsely elevated serum PSA concentrations, due to prostatitis and urinary retention, are well recognized.[73–76] Stamey found that transurethral resection of the prostate causes a 53-fold increase in the immediate post-operative PSA serum levels.[71] However it should be remembered that these findings were obtained using the Tandem-R PSA or Pros-Check PSA assay kits. Due to lack of data from a relatively large number of patients, the effects of these factors in Japanese patients investigated using Eiken PSA or Markit-F PA kits still require confirmation.

CLINICAL IMPORTANCE OF PSA IN THE DETECTION OF PROSTATE CANCER

Following the initial clinical investigation performed by researchers at the RPMI, clinical studies to assess the significance of PSA were begun. Kuriyama et al. of RPMI,[61] using their original EIA, were unable to detect PSA in sera from female patients with various types of cancer. The mean serum PSA and its standard deviation in normal males and males with non-prostatic cancers were similar. As mentioned above, 68% of BPH cases exhibited elevated PSA values, which implies a low specificity for PSA in the detection of prostate cancer. However, there was a statistically significant difference between the serum PSA values in BPH and prostate cancer. Kuriyama et al. showed that simultaneous determination of PSA and PAP offers an increased sensitivity (51% PSA in a single assay compared with 80% in combination) and high specificity (90%) for the diagnosis of prostate cancer. As a cut-off value for PSA, they used a mean PSA + 2SD in BPH cases.[77] Following the observation of Papsidero et al.[48] that the molecular weight of PSA in sera was about 100,000, Chu and Kuriyama found a binding protein for PSA in the sera of patients with prostate cancer. This protein was purified by affinity chromatography and found to belong to the IgG_2 family.[78] Patients with stage D prostate cancer have been shown to have significantly high levels of binding protein using reversed EIA for the PSA determination.[79] This protein was later found to be ACT.

Using Tandem-R PSA or Pros-Check PSA assays, numerous reports have been published evaluating serum PSA as a means of screening or diagnosing prostate cancer.[62–63,69,73,80–96] Recently, a new EIA has been developed in the USA (IMx Dainapack, Abbott, Chicago, USA) and evaluated there and in Japan.[97–98] Japanese researchers have also published many studies assessing the clinical evaluation of PSA, mainly using Eiken PSA, Markit-F PA and other assay kits.[64–65,98–132]

Normal values in males of 4.0 ng/ml and 2.5 ng/ml have been established for the Tandem-R PSA[123] and Pros-Check PSA assays,[34,123] respectively. Of the healthy males studied, 97% had PSA values <4.0 ng/ml in the Tandem-R PSA assay and the normal value

in the Pros-Check assay was taken to be the mean + 2SD (about 95% of patients). However, since it is well known that serum PSA values measured by the Pros-Check assay are usually higher than those obtained by the Tandem-R assay, these "reversed" normal values are strange. When we evaluated serum PSA in normal Japanese age-matched males (age: > 50 years), we obtained for the mean + 3SD of the serum PSA a value of 1.9 ng/ml in the Tandem-R PSA assay[124] and 3.2 ng/ml in the Pros-Check PSA assay.[117] In addition, the normal value of serum PSA in Japanese and European patients is similar using the Delfia PSA assay kit (Pharmacia Biotech AB, Uppsala, Sweden).[118] Thus, for any discussion or decision involving the normal value of serum PSA, racial differences as well as assay methods need to be considered. Irrespective of which assay method or normal value is used, at least 1–5% of asymptomatic males have an elevated serum PSA because PSA is an antigen which is prostate tissue-specific but not cancer-specific.

In clinical practice, distinction between BPH and prostate cancer is very important when using PSA as a tumor marker. Table 2 summarizes the positive rates obtained with serum PSA for these 2 groups of patients, from data gathered from a relatively large number of studies performed in the USA and Japan.[65,98,117–118,120,124–127] As shown in Table 2, if normal values were used as cut-off points, 24.5–75.0% of BPH cases exhibit elevated serum PSA levels. In addition, the differences in the positive rates, shown by the PSA determinations, between BPH and stage A or B prostate cancer are variable: 6–36.9%. The positive rates in the advanced stages were almost 100%. Thus, serum PSA increases with the progression of both the clinical and pathological stages. Compared with the results obtained in the USA, prostate cancer is characterized in Japan by the low specificity (high-false positive rate for BPH) and relatively high sensitivity for diagnosis for organ-confirmed stages.[6] This may be due to the lower cut-off point chosen.

As a cause of false positives in BPH cases other than the characteristics of the PSA tissue-specificity, the volume of prostatic tissue is thought to play a part, reflecting serum PSA values.[94,107,117] Stamey et al.[94] have calculated that benign hyperplastic tissue elevates the serum PSA level at a rate of 0.3 ng/ml/gm BPH tissue (Pros-Check PSA). However, Weber et al.[128] were unable to demonstrate such a close relationship between the amount of BPH tissue and serum PSA concentration and observed a glandular component with PSA giving rise to varying serum PSA levels. Another factor contributing to elevated serum PSA in cases of BPH is thought to be coexisting prostatic intraepithelial neoplasia. Brawer[61] and Lee et al.[129] found a proportional increase in serum PSA with coexisting intraepithelial neoplasia.

As another cause of overlapping serum levels of PSA between patients with BPH and those in the early stages of prostate cancer, the characteristics of PSA assay systems themselves must be considered: detection limits, standards used, antigen sites of each anti-PSA antibody, among others. Indeed, Horton et al.[130] and Akimoto et al.[109] have found that differences in clinical data between the Tandem-R PSA and Pros-Check PSA assays depend upon a difference in PSA expression by the standard. Moreover, Arai et al.[131] demonstrated that lymph node involvement could be suggested by a combination of serum PSA values (Markit-F PA) and Gleason score. This finding was not confirmed by the determination of PSA with the Tandem-R PSA assay. Table 3 shows the serum PSA values used to obtain various degrees of specificity, with the use of BPH cases as controls. This was per-

Table 2. Serum PSA levels in patients with BPH and localized prostate cancer.

Assay methods	Cut-off value	No of cases			Positive rate (%)		
		BPH	Prostate cancer		BPH	Prostate cancer	
			Stages A/B	Stage C		Stages A/B	Stage C
Tandem-R PSA[a]	4.0	597	319		24.5	57.4	
Markit-F PA	3.8	111	33	22	26.1	63	95
Eiken PA	3.0	116	22	21	50.8	68	100
Pros-Check PSA	3.2	132	27	23	75.0	83	96
Delfia PSA	2.0	126	33	30	66.7	79	97
Ball ELSA	2.0	125	33	30	67.2	79	97
ACS-PSA	2.3	68	19	11	62	68	91
Tandem-R PSA	1.9	149	29	28	68.4	83	96
Markit-M PA	1.8	137	32	22	34.3	67	93
IMx PA	4.0	112	32	46	41.1	81	93

[a] *Summaries of the data from Lange, Hudson and Partin (Ref #92, 125, 126).*

Table 3. Serum PSA levels determined with various degrees of specificity.

Specificity (%)	PSA assay systems: PSA (ng/ml)							
	Markit-F	Eiken	Tandem-R	Pros-Check	Delfia	Ball ELSA	ACS	Markit-M
90	5.5	8.1	21	32	16	18	17	4.6
80	4.2	5.9	9.0	19	9.2	11	9.6	2.9
70	3.4	4.3	6.2	12	6.3	6.7	7.9	2.0
60	2.6	3.6	4.6	8.9	4.9	5.4	6.3	1.2
50	2.1	3.0	3.6	5.9	3.7	3.5	3.8	0.9

formed using almost the same Japanese patients as in the investigation of the different assays. Compared with the normal value in each assay system listed in Table 2, the serum PSA values exhibit a high degree of specificity. However, some kits such as Markit-F, Eiken PSA and Markit-M which were originally made in Japan do not show such a marked specificity (80 or 90%) compared with the normal value. In addition, another suitable cut-off value for the differential diagnosis of prostate cancer from BPH needs to be established. The best results were obtained with values of 9–13 ng/ml in the Tandem-R PSA assay[126] as found in the USA[122] and 3.6 ng/ml in the Markit-M PA assay.[120] Sensitivities for the diagnosis of stages A/B and C prostate cancer are presented in terms of various degrees of specificity in Table 4. Thus, using PSA alone, the detection or screening for the early stages of prostate cancer with a high degree of specificity is still limited.

Although, as initial studies recognized,[77] the simultaneous determination of PSA and PAP has very little effect in improving the efficacy of prostate cancer detection,[117] only in a few cases of prostate cancer is the PSA negative and PAP positive. In an attempt to increase the clinical efficiency of the diagnosis of prostate cancer, Cooner et al.[133] found a combination of PSA detection and DRE to be useful.

The clinical significance of serum PSA for the diagnosis of prostate cancer can be summarized as follows: (1) Normal values differ for each assay method; (2) Prostate cancer patients have significantly elevated serum PSA levels which increase as the disease progresses both clinically and pathologically; (3) Some cases of BPH also exhibit mild to moderately high serum PSA levels due to the prostate volume or coexisting intraepithelial neoplasia; (4) To obtain an effective diagnosis, cut-off points need to be modified used in combination with DRE; and (5) As mentioned by Chybowski et al.[133] and Aral et al.,[131] serum PSA measurement reduces the need for bone scintigraphy or helps in the recognition of lymph node involvement. Although there are some limitations and careful analysis of the data is necessary, it is clear that PSA measurement is the best tool for the diagnosis of prostate cancer we have, compared with other methods such as DRE and transrectal ultrasonography. However, we must have sufficient information about the characteristics of the assay used to determine PSA, before a meaningful analysis of the data can be made.

Table 4. Sensitivities of PSA determinations at various degrees of specificity.

Specificity (%)	Stages in prostate cancer	PSA assay systems; sensitivities (%)							
		Markit-F	Eiken	Tandem-R	Pros-Check	Delfia	Ball ELSA	ACS	Markit-M
90	A + B	44	27	28	26	36	30	32	39
	C	82	66	76	78	83	83	91	79
80	A + B	56	41	45	37	42	45	53	56
	C	95	95	75	87	90	90	91	90
70	A + B	68	45	59	44	58	55	53	61
	C	95	95	89	87	93	90	91	93
60	A + B	72	55	59	52	58	58	58	69
	C	95	95	89	87	93	93	91	95
50	A + B	81	68	71	59	64	67	58	78
	C	95	100	89	87	97	97	91	95

PSA AS A FOLLOW-UP MARKER IN PATIENTS WITH PROSTATE CANCER

The initial investigations were performed at the RPMI. Kuriyama et al.[134] demonstrated that serum PSA levels during the pre-chemotherapeutic period correlated with prognosis in 96 patients with advanced prostate cancer who died during the observation period. In addition, there was a trend towards longer survival in those patients with lower initial PSA values. The possible value of serum PSA was established as a means of monitoring prostate cancer in 19 patients with metastatic disease and 34 patients who had received curative therapy for localized disease. Of the 19 prostate cancer patients, 14 (74%) cases involved PSA analysis of 232 samples and the clinicopathological evaluation of treatment response. In another study, a total of 161 serum samples were analyzed for PSA during a period of 12–114 weeks. Five patients developed metastasis and all exhibited increasingly elevated PSA levels. An elevated serum PSA was detected 68 weeks before the recurrence of the disease was clinically diagnosed in 1 patient.[134–136]

In the USA and Europe, clinical evaluations of serum PSA as a follow-up marker have been carried out.[95,137–158] Generally speaking, serial PSA levels were found to be the best reflection of disease status. For example, PSA levels were elevated at relapse or progression, fell or returned to normal during successful treatment, and rose in the presence of a residual tumor or unsuccessful therapy, irrespective of the nature of the treatment such as total prostatectomy,[141,143–148,156] irradiation therapy,[142,144,149,150] and endocrine treatment.[139–140,145,146,151–153,155,158] Serum PSA could be detected several weeks after radical prostatectomy due to its long half-life.[126] After becoming undetectable, if the PSA rose to detectable levels again, 100% of such patients relapsed 3–6 months later.[126] A correlation between high or increasing serum PSA levels and disease progression was found in radiation-treated patients by Stamey et al.[142] and Kaplan et al.[150] However, as noted by Goldrath and Messing and Leo et al., it must be remembered that there are cases where the serum PSA levels do not increase on disease progression[147] or where PSA has not identified some cases with stage D2 prostate cancer, treated by hormonal therapy.[157]

As in the USA and Europe, there are several Japanese reports evaluating PSA as a tool for follow-up during anti-androgen therapy.[115,117–118,120,131,159–161] For example, Akimoto et al.[160] found no relationship between patient prognosis and the serum PSA level before initiation of treatment. However, when the patients were divided into 2 groups, those who had normal levels of serum PSA 1–6 months after treatment and those who did not, there was a statistically significant difference in prognosis between the groups. The authors also found a statistically significant correlation between the patient prognosis and the minimum level of serum PSA observed. Shinoda[161] observed the serum PSA fall to about half the pretreatment value 7 days after initiation of successful endocrine therapy. Then he divided his patients into 2 groups, those who did or did not achieve a level of less than 50% of their initial serum PSA at 7 days, and found a statistically significant correlation with no progression of disease (Fig. 1). This data suggests that serial serum PSA levels are strongly correlated with the clinical response and are helpful for establishing the prognosis of prostate cancer patients treated with endocrine therapy. Recently, there have been some reports in which serum PSA changed in parallel with serum testosterone levels.[156–157,162] Therefore, in assessing the significance of serum PSA levels in patients treated with endocrine therapy, such effects need to be taken into account. Almost all patients relapsing or progressing had elevated PSA levels.[117–118,120,131,159–161] Kuriyama et al.[119] have proposed criteria for evaluating prostatic tumor markers during follow-up (Table 5). Using these criteria, the clinical usefulness of various assay kits for PSA, γ-Sm

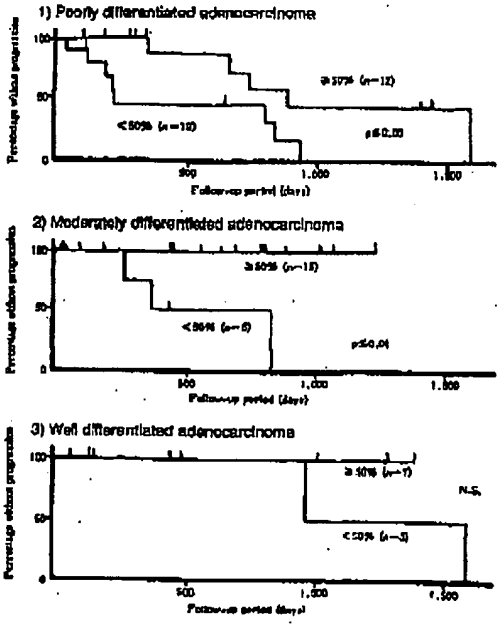

Fig. 1. Percentage without progression, according to the reduction in serum PSA on the 7th day.

Table 5. Criteria for evaluating tumor markers in patients with prostate cancer on follow-up.

Response	Responders (CR, PR & SI)	Nonresponders (PD)
Score 4	Abnormal → Normalizing	Normal → Abnormalizing before diagnosis
	Abnormal → Decreasing > 1/2 within 7 days	
3	Abnormal → Decreasing > 1/2	Normal → Abnormalizing at diagnosis
		Abnormal → Increasing
2	Abnormal → Decreasing < 1/2	Abnormal → Abnormal
	Normal → Normal	Normal → Abnormalizing after diagnosis
1	Abnormal → No decrease	Abnormal → Decreasing
	Normal → Increasing	Normal → Normal

and PAP was evaluated (Table 6).[124] Matching rates (score; ≥ 3) and mean scores were higher for PSA than those for γ-Sm or PAP, again objectively confirming the superiority of PSA.

PSA AS AN IMMUNOHISTOCHEMICAL MARKER OF PROSTATE NEOPLASM

Nadi et al.[163] in the USA and Okano et al.[166] in Japan using the same antibody (p17)[20] from the same source (RPMI) performed immunohistochemical studies on PSA. Based upon data obtained in later studies,[163–169] 99% of both primary and metastatic prostatic lesions stained positive for PSA while the corresponding figure in non-prostatic tissue was 0%. Therefore, PSA staining could be used to detect adenocarcinoma metastasized from an unknown origin.[167,169–170] Moreover, Deguchi et al.[171] detected micrometastasis to lymph node and bone by applying the polymerase chain reaction technique to PSA. A limitation of the immunohistochemical staining of PSA was the finding of weak staining in poorly differentiated carcinoma[165,169,172] in spite of other evidence[167] and some slight cross-reactivity with tissues other than the prostate.[173] As far as a correlation between PSA staining and disease progression is concerned, Epstein and Eggleston[166] found only a small area staining weakly for PSA in stage A2 prostate cancer which correlated with disease progression. Okano[174] also found that patients with poorly differentiated carcinoma, exhibiting no PSA staining, had a poorer prognosis.

RECENT ADVANCES IN THE APPLICATION OF PSA TO THE DIAGNOSIS AND SCREENING OF PROSTATE CANCER

Various attempts have been made to improve the detection rate of prostate cancer, especially in its early stages. Serum PSA levels in asymptomatic healthy males have been found to correlate with prostate volume and age.[175–179] Labrie et al.[176] have found a correlation coefficient of $r = 0.184$ in benign cases and $r = 0.398$ in prostate cancer. Therefore, the "normal value" of PSA changes with age (Age-specific PSA reference) and the degree of change of serum PSA may predict the occurrence of prostate cancer (PSA velocity).[175] Furthermore, following Babarian's observations,[179] Benson et al.[180–181] evaluated serum PSA levels divided by prostatic volume (PSA density, PSAD) for the differential diagnosis of prostate cancer

Table 6. Clinical usefulness of tumor markers in the follow-up of prostate cancer patients.

Tumor maker & detection methods	PSA									PAP
	Tandem-R	Markit-F	Markit-M	Elken	Pros-Check	Delfia	CIS	ACS	γ-Sm	
Responder										
No of cases	30	10	9	28	10	10	10	5	29	30
Cases score ≥ 3	30	10	9	26	10	10	10	5	18	17
(%)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(62)	(57)
Mean score	3.5	3.9	3.8	3.6	3.4	3.4	3.4	3.4	3.0	3.0
Non-responder										
No of cases	13	5	4	13	3	5	4	4	12	12
Cases score ≥ 3	10	5	4	10	2	5	4	4	8	7
(%)	(77)	(100)	(100)	(77)	(67)	(100)	(100)	(100)	(67)	(58)
Mean score	2.9	3.2	3.3	2.8	3.0	3.2	3.3	3.3	2.5	2.3
Total										
No of cases	43	15	13	41	13	15	14	9	41	42
Cases score ≥ 3	40	15	13	36	12	15	14	9	26	24
(%)	(93)	(100)	(100)	(88)	(92)	(100)	(100)	(100)	(63)	(59)
Mean score	3.3	3.7	3.6	3.3	3.3	3.3	3.4	2.9	2.9	2.8

Table 7. Serum PSA-α₁-antichymotrypsin complex levels in various diseases.

Disease	No of examination	PSA-ACT (ng/ml)		Student's t test		No of positive cases (%)		
		Range	Mean ± SD	vs NHS	vs BPH	≥ 0.61	≥ 3.5	≥ 5.4*
Normal males	198	<0.10 – 1.0	0.182 ± 0.140	–		3 (1.5)	0	0
Normal females	19	<0.10 – 0.50	0.189 ± 0.129	NS		0	0	0
RCC/BT	60	<0.10 – 3.4	0.600 ± 0.741	<0.0001		15 (25)	0	0
Acute prostatitis	4	1.6 – 3.2	2.40 ± 0.616	<0.0001	NS	4 (100)	0	0
BPH	91	<0.10 – 10	1.48 ± 1.95	<0.0001	–	55 (60)	9 (10)	6 (7)
Prostate cancer	79	<0.10 – 4200	169 ± 569	<0.0001	<0.01	68 (86)	55 (70)	54 (68)
Stage A	12	<0.10 – 92	9.34 ± 26.2	<0.0001	<0.005	7 (58)	2 (17)	2 (17)
Stage B	18	0.30 – 48	11.5 ± 16.4	<0.0001	<0.0001	11 (85)	5 (38)	5 (38)
Stage C	18	<0.10 – 410	71.9 ± 115	<0.0001	<0.0001	16 (89)	15 (83)	15 (83)
Stage D	36	0.10 – 4200	314 ± 618	<0.0001	<0.0005	34 (94)	33 (92)	32 (89)
WEL	13	<0.10 – 310	33.6 ± 84.1	<0.0001	<0.0005			
MOD	17	0.20 – 470	63.1 ± 143	<0.0001	<0.0001			
POR	18	1.2 – 2800	233 ± 642	<0.0001	<0.001			

* *0.61 ng/ml (mean + 3SD) in normal males and 3.5 and 5.4 ng/ml (mean + SD and + 2SD) in BPH patients respectively.*

Table 8. Characteristics of assays for measuring serum PSA levels.

PSA assays	Makit-F PA	Elken PSA	Tandem-R PSA	Pros-Check PSA	Delfia PSA	Ball ELSA PSA	ACS PSA	Makit-M PA
Produced in	Japan	Japan	USA	USA	Sweden	France	USA	Japan
Principle	EIA competition	RIA double-antibody	IRMA sandwich	RIA double-antibody	TRFIA sandwich	IRMA one-step sandwich	CLIA one-step sandwich	EIA one-step sandwich
1st Ab	PoAb	PoAb	MoAb	PoAb	MoAb	MoAb	MoAb	MoAb
2nd Ab	anti rabbit-IgG	anti rabbit-IgG	MoAb	anti rabbit-IgG	MoAb	MoAb	MoAb	MoAb
Tracer	β-Gal	I^{125}	I^{125}	I^{125}	Eu	I^{125}	CL	HRP
Detection range (ng/ml)	1.6–300	1.0–100	0.2–100	0.6–50	0.1–500	0.2–130	0.13–260	0.1–100

and BPH. There was a statistically significant difference in PSAD between BPH and prostate cancer. This observation was confirmed by Lee et al.[182] As a critical cut-off point for PSAD, a value of 0.20 is recommended. Further work in this area is now being carried out, especially in Japan.

As PSA is a serine protease,[52–55,183] serum PSA must be conjugated to a protease inhibitor for neutralization. Stenman et al.[184] and Lilja et al.[185] independently found that PSA reacts with α_1-antichymotrypsin (ACT) in serum and the resulting complex is the major form in which PSA is present in the serum of patients with prostate cancer. The same groups[184–185] and Wood et al.[186] have established immunoassays for the determination of the PSA-ACT complex and evaluated its use in differentiating prostate cancer from BPH. Their results have prompted a search for a specific indicator for prostate cancer. In Japan, Kuriyama et al.[188] have developed an RIA for PSA-ACT and are evaluating its clinical significance. Preliminary results indicate that serum PSA-ACT levels, even in stage A prostate cancer, are significantly higher than in BPH (Table 7). In addition, Christensson et al.[189] have used a specific monoclonal antibody and reported that free PSA is a more sensitive indicator of prostate cancer. The assay kit for γ-Sm detects only the free form of PSA[188] and, therefore, Demura et al.[190] have evaluated the PSA/γ-Sm ratio for the detection of prostate cancer. They have recommended a PSA/γ-Sm ratio of 1.45 as the most effective cut-off point for differentiating between prostate cancer and BPH. Thus, γ-Sm is now being reevaluated for assaying the free form of PSA.

STANDARDIZATION OF PSA ASSAYS

As mentioned above, there are more than 10 assay kits available in Japan. In Table 8, details of 8 are summarized. Each of them has different characteristics such as the sensitivity of detection, working range, antibody

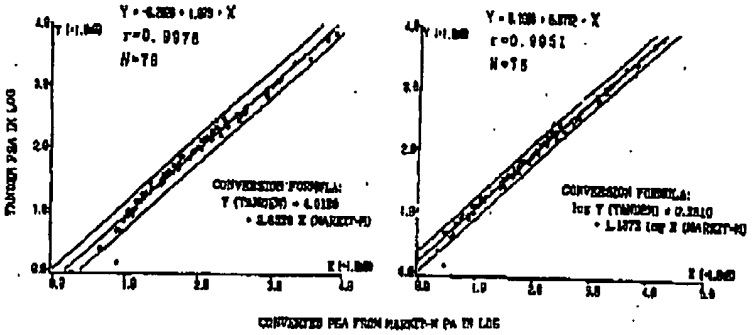

Fig. 2. Conversion between PSA assays, from Markit-M PA to the Tandem-R PSA in prostate cancer cases.

used (whether monoclonal or polyclonal), antigen determining site, RIA or non-isotopic method, and tracer used. Not surprisingly, this tends to confuse urologists when analyzing the data. The difficulty in comparing data from the Tandem-R PSA and Pros-Check PSA assays has been described by Graves et al.[191] They pointed out the need for an international PSA standard as early as 1990.[191] To this end, Stamey organized an International Conference for PSA Standardization at Stanford in 1992 which recommended the p30 of Sensabaugh[40] as the world PSA standard because p30 has been shown to be identical to PSA.[39] A second conference is scheduled at Stanford for Summer 1994.

In another attempt to standardize PSA assays, The Japanese Urological Association (JUA) set up a subcommittee to examine the possibility of comparing data in Japanese studies.[192] Using 142 sera from BPH and prostate cancer cases, the group successfully produced formulae for comparison using a linear regression technique. These conversion formulae were then evaluated in practice. Serum PSA levels were simultaneously measured by Tandem PSA and Markit-M PA (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) or Eiken PSA kits in patients with BPH and prostate cancer; only data above the limit of detection were used in this comparison. High correlations between the measured and converted Tandem-R PSA values were observed. Correlation coefficients ranged from 0.8758 in the case of the Eiken PSA kit converted using a logarithmic scale in the BPH cases (n = 58) to 0.9978 for the Markit-M PA kit in prostate cancer cases (n = 76). The 95% of confidence limits of the linear regression curves were very narrow in the case of the Markit-M PA kit for BPH (n = 63) and prostate cancer (Fig. 2) and for the Eiken PSA kit in prostate cancer patients (n = 101). These data suggest that the converting formulae proposed by the JUA for the standardization of various PSA assays have a practical use.

CONCLUSIONS

PSA is a glycoprotein with a molecular weight of 33,000–34,000 and it has a kallikrein-like, serine protease activity. It is exclusively produced by the epithelial cells of the prostate, regardless of tissue type, and hence can be regarded as a prostate tissue-specific protein. Approximately 25–75% of patients with BPH have elevated serum PSA levels depending upon the type of assay kit or normal range used. Therefore, it is unlikely that PSA by itself will become an effective screening tool for the diagnosis of the early stages of prostate cancer. However, by using a recently developed assay method involving PSAD, PSA-ACT complex and the free form of PSA, it may be possible to make a differential diagnosis between prostate cancer and BPH. PSA is an extremely sensitive tumor marker for monitoring patients after therapy.

ACKNOWLEDGMENTS

The author wishes to express his profound gratitude to Professors Yukimichi Kawada of Gifu University, Gifu, Japan, Osamu Yoshida of Kyoto University, Kyoto, Japan, and Jun Shimazaki of Chiba University, Chiba, Japan for their encouragement to continue this work and for their own contribution. Emeritus Professors Keizo Shida of the Prostate Research Foundation, Tokyo, Japan and Yoshio Aso of Tokyo University, Tokyo, Japan are also thanked for their support.

REFERENCES

1. Wynder EL, Fujita Y, Harris R, Hirayama T, Hiyama T. Comparative epidemiology of cancer between the United States and Japan. Cancer 1991;67:746–763
2. Shida K, Akimoto S, Kuriyama M, Arai Y. Free-type prostate tumor markers. Medical Tribune 1993; November 4

3. Yatani R, Kusumo I, Shiraishi T, Hayashi T, Stemmermann GN. Latent prostatic carcinoma: Pathological and epidemiological aspects. Jpn J Clin Oncol 1989;19:319–326
4. Scher HI. Prostatic cancer: Are we closer to rational treatment selection? Curr Opin Oncol 1992;4:442–454
5. Garnick MB. Prostate cancer: Screening, diagnosis, and management. Ann Intern Med 1993;118:804–818
6. Cooner WH. Prostate cancer: New hope for early diagnosis. Ala Med 1987;56:13–16
7. DeWys WD. Detection, staging, and monitoring of prostate cancer: Clinical overview and opportunities for research. Prog Clin Biol Res 1987;239: 613–630
8. Murphy GP. Prostate cancer 1986: Where have we been and where are we going? Prog Clin Biol Res 1987;237:1–3
9. Mueller BJ. Cancer of the prostate. Do yearly digital rectal examinations save life? Postgrad Med 1989;86:115–119
10. Lange PH. Controversies in management of apparently localized carcinoma of prostate. Urology 1989;34,suppl 4:13–18
11. Chodak GW. Early detection and screening for prostatic cancer. Urology 1989;34,suppl 4:10–12
12. Denis LJ. To screen or not to screen? Prostate 1992;suppl 4:63–70
13. Gerber GS and Chodak GW. Value of prostate cancer screening. Eur Urol 1993;24:161–165
14. Bahnson PR. Treatment of stage A1 prostate cancer: The case for treatment. Semin Urol 1993;11:54–57
15. Austenfeld MS. Treatment of stage A1 prostate cancer: The case for observation. Semin Urol 1993;11: 58–63
16. Imai K, Yamanaka H. Screening examination for prostate cancer. Early detection and mass screening. Jpn J Urol 1993;84:1175–1187
17. Gutman EB, Sprough BB, Gutman AB. Significance of increased phosphatase activity of bone at site of osteoplastic metastasis secondary to cancer of prostatic gland. Am J Cancer 1936;28:485–495
18. Schulman S, Mamrod L, Gonder MJ, Sianes WA. The detection of prostatic acid phosphatase by antibody reaction in gel diffusion. J Immunol 1964;93:474–480
19. Cooper JF, Foti AG. A radioimmunoassay for prostatic acid phosphatase; I. Methodology and range of normal male serum values. Invest Urol 1974;12:98–103
20. Vihko P, Sanganti E, Janne O, Peltonenn L, Vihko R. Serum prostate-specific acid phosphatase; Development and validation of a specific radioimmunoassay. Clin Chem 1978;24:1915–1919
21. Chu Tm, Wang MC, Scott WW, Gibbon DE, Schmidt JD, Loeming SA, Prout GR, Murphy GP. Immunochemical detection of serum prostatic acid phosphatase; Methodology and clinical evaluation. Invest Urol 1978;15:319–322
22. Choe BK, Pontes EJ, Marrison MK, Rose NR. Human prostatic acid phosphatase. II. A double-antibody radioimmunoassay. Arch Androl 1978;1:227–233
23. Lee CL, Wang MC, Murphy GP, Chu TM. A solid-phase fluorescent immunoassay for human prostatic acid phosphatase. Cancer Res 1978;38:2871–2878
24. Griffiths JC. Prostate-specific acid phosphatase: Re-evaluation of radioimmunoassay in diagnosing prostatic disease. Clin Chem 1980;26:433
25. Masukagami T, Ando K, Shimazaki J. Prostatic acid phosphatase by enzyme immunoassay. Acta Urol Jpn 1984;30:1691–1695
26. Rubenstein M, Guinan PD, McKiel CF, Dubin A. Review of acid phosphatase in the diagnosis and prognosis of prostatic cancers. Clin Physiol Biochem 1988;6:241–252
27. Kuriyama M, Takahashi T, Kawada Y. Clinical significance of prostatic acid phosphatase assay as a tumor marker. Nihon Rinsyo 1990;suppl:925–933
28. Konturri M. Is acid phosphatase (PAP) still justified in the management of prostate cancer? Acta Oncol 1991;30:169–170
29. Wang MC, Valenzuela LA, Murphy GP, Chu Tm. Purification of a human prostate specific antigen. Invest Urol 1979;17:159–163
30. Papsidero LD, Wang MC, Kuriyama M, Horoszewicz J, Leong SS, Croghan GA, Valenzuela LA, Murphy GP, Chu TM. Human prostate epithelial cell-specific antigen. In: Murphy GP (ed) The Prostatic Cell: Structure and Function, Part A. New York: Alan R. Liss, Inc., 1981;435–443
31. Wang MC, Kuriyama M, Papsidero LD, Loor RM, Valenzuela LA, Murphy GP, Chu TM. Prostate antigen of human cancer patients. In: Busch H, Yeoman LC (eds) Methods in Cancer Research. New York: Academic Press, 1982;179–197
32. Kuriyama M, Loor R, Wang MC, Lee CL, Killian CS, Papsidero LD, Inaji H, Nishiura T, Slack NH, Murphy GP, Chu TM. Prostatic acid phosphatase and prostate-specific antigen in prostate cancer. In: Murphy GP (ed) International Advances in Surgical Oncology. New York: Alan R. Liss, Inc, 1882;29–49
33. Chu TM, Wang MC, Lee CL, Killian CS, Kuriyama M, Papsidero LD, Valenzuela LA, Murphy GP. Prostate cancer markers. In: Sell S, Warren B (eds) Human Cancer Markers. Clifton: Humana Press, 1982;179–190
34. Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987;317:909–916
35. Prostate-specific antigen. Lancet 1988;8602:318–319
36. Walsh PC, Oesterling JE, Epstein JI, Bruzek DJ, Rock RC, Chan DW. The value of prostate-specific antigen in the management of localized prostate cancer. Prog Clin Biol Res 1989;303:27–33
37. Lange PH, Brawer MK. Serum prostate-specific antigen: Its use in diagnosis and management of prostate cancer. Urology 1989;33,suppl 6:13–17
38. Killian CS. Prostate-specific antigen: A seroprognosticticator for progression of disease in patients with prostatic cancer. In: Catalona WJ, Coffy DS, Karr JP (eds) Clinical Aspect of Prostate Cancer. New York: Elsevier Science Publishing Co., 1989;128–146
39. Brawer MK. Prostate specific antigen. A review. Acta Oncol 1991;30:161–168
40. Oesterling JE. Prostate specific antigen: A critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 1991;145: 907–923
41. Akimoto S, Masai M, Kitagawa K, Shimazaki J. Tumor marker in the urological tumor: Its reliability and limitation: PSA. Urol Surg 1991;1065–1072

42. Sell S. Detection of cancer by tumor markers in the blood: A review to the future. Crit Rev Oncogenesis 1993;4:419–433
43. Flocks RH, Urich VC, Patel CA, Opitz JH. Studies on the antigenic properties of prostate antigen. J Urol 1960;84:134–143
44. Ablin RJ, Bronson P, Soanes WA, Witebsky E. Tissue and species-specific antigens of normal human prostatic tissue. J Immunol 1970;104:1329–1339
45. Li TS , Beling CG. Isolation and characterization of two specific antigens of human seminal plasma. Fertil Steril 1973;24:134–137
46. Sensabaugh GF. Isolation and characterization of a semen-specific protein from human seminal plasma: A potential new marker for semen identification. J Forensic Sci 1978;23:106–109
47. Wang MC, Valenzuela LA, Murphy GP, Chu TM. A simplified purification procedure for human prostate antigen. Oncology 1982;39:1–5
48. Papsidero, LD, Wang MC, Valenzuela LA, Murphy GP, Chu TM. A prostate antigen in sera of prostate cancer patients. Cancer Res 1980;40:2428–2432
49. Chu TM, Kawinski E, Hibi N, Croghan G, Wiley J, Killian CS, Corral D. Prostate-specific antigenic domain of human prostate-specific antigen identified with monoclonal antibodies. J Urol 1989;141:152–156
50. Riegman PHJ, Vlietstra RJ, van der Korpert JAGM, Romijin JC, Trapman J. Characterization of the prostate-specific antigen gene: A novel human kallikrein-like gene. Biochem Biophys Res Commun 1989;159: 95–102
51. Schedlich LJ, Bennetts BM, Morris BJ. Primary structure of a human glandular kallikrein gene. DNA 1987;6:429–437
52. Lilja H. A kallikrein-like serine protease in prostatic fluid leaves the predominant seminal vesicle protein. J Clin Invest 1985;76:1899–1903
53. Ban Y, Wang MC, Watt KW, Loor R, Chu TM. Proteolytic activity of human prostate-specific antigen. Biochem Biophys Res Commun 1984;123:482–488
54. Hara M, Koyanagi Y, Inoue T, Fukuyama T. Some physico-chemical characteristics of γ-seminoprotein, an antigenic component specific for human seminal plasma. Jpn J Legal Med 1971;25:322–324
55. Eto K, Kawai T, Ishi M, Okura H, Ohmori H, Saito A, Shimazaki J, Sonoda T, Tsuchida M, Niijima T, Nishiura T, Hara M, Machida T, Matsumoto K, Yamamaka H, Yonese Y. Clinical evaluation of measurement of serum concentration of γ-seminoprotein (γ-Sm) for diagnosis of prostatic cancer. Jpn J Urol 1985;76:1936–1842
56. Kuriyama M, Takeuchi T, Shinoda I, Okano M, Nishiura T. Clinical evaluation of γ-seminoprotein in prostate cancer. Prostate 1986;8:301–311
57. Siddall JK, Shetty SD, Cooper EH. Measurement of serum γ-seminoprotein and prostate specific antigen evaluated for monitoring carcinoma of the prostate. Clin Chem 1986;32:2040–2043
58. Shimazaki J, Akimoto S, Akakura K, Yoshiki T, Yoshida O, Okada K, Matsuoka K, Noda S, Eto K. Serum γ-seminoprotein determination in prostate cancer. Acta Urol Jpn 1991;37:341–347
59. Sensabaugh GF, Blake ET. Seminal plasma protein p30: Simplified purification and evidence for identity with prostate specific antigen. J Urol 1990;144:1523–1526
60. Graves HCB, Kamarel M, Stamey TA. Identify of prostate specific antigen and the semen protein p30 purified by a rapid chromatography technique. J Urol 1990;144:1510–1515
61. Kuriyama M, Wang MC, Papsidero LD, Killian CS, Shimano T, Valenzuela LA, Nishiura T, Murphy GP, Chu TM. Quantitation of prostate-specific antigen in serum by a sensitive enzyme immunoassay. Cancer Res 1980;40:4658–4662
62. Liedtke RJ, Batjer JD. Measurement of prostate-specific antigen by radioimmunoassay. Clin Chem 1984; 30:649–652
63. Killian CS, Yang N, Emrich LJ, Vargas FP, Kuriyama M, Wang MC, Slack NH, Papsidero LD, Murphy GP, Chu TM, the Investigators of the National Prostatic Cancer Project. Prognostic importance of prostate-specific antigen in patients with stage B2 to D1 prostate cancer. Cancer Res 1985;45:886–891
64. Miki M, Machida T, Yanagisawa M, Yoshida S, Yamazaki H, Konda N, Azuma Y, Takahashi T, Kurauchi Y. Clinical evaluation of serum prostatic specific antigen measurement by radioimmunoassay. Jpn J Urol 1984;75:1982–1988
65. Kuriyama M, Shinoda I, Takeuchi T, Ban Y, Kawada Y, Nishiura T, Yoshiki T, Okada K, Yoshida O, Hamami G, Kamidono S, Mita T, Terasoma K. Clinical evaluation of prostate-specific antigen with an HIA; A co-operative study. Nishinihon J Urol 1987;49: 1431–1438
66. Oesterling JE, Chan DW, Epstein JI, Kimball AW Jr, Bruzek DJ, Rock RC, Brendler CB, Walsh PC. Prostate specific antigen in the preoperative and postoperative evaluation of localized prostatic cancer treated with radical prostatectomy. J Urol 1988;139: 763–765
67. Dejter SW Jr, Martin JS, McPherson RA, Lynch JH. Daily variability in human serum prostate-specific antigen and prostatic acid phosphatase: A comparative evaluation. Urology 1988;32:288–292
68. Maatman TJ. The role of prostate specific antigen as a tumor marker in men with advanced adenocarcinoma of the prostate. J Urol 1989;141:1378–1380
69. Adjiman S, Zerbib M, Brochard M, Duguc MA, Debre B, Steg A. Does rectal examination modify the blood level of specific prostatic antigen? Ann Urol (Paris) 1989;23:291–294
70. Brawer MK, Schiffman RB, Ahmann FR, Ahmann MB, Coulis. The effect of digital rectal examination on serum levels of prostate-specific antigen. Arch Path Lab Med 1988;112:1110–1102
71. Stamey TA. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. Monogr Urol 1989;10:49
72. Lee F, Littrup PJ. The role of digital rectal examination, transrectal ultrasound, and prostate specific antigen for the detection of confined and clinically relevant prostate cancer. J Cell Biochem Supp 1992;16H:69–73
73. Seamonds B, Yang N, Anderson K, Whitaker B, Shaw LM, Bollinger JR. Evaluation of prostate-specific antigen and prostatic acid phosphatase as prostate cancer markers. Urology 1986;28:472–479
74. Armitage TG, Cooper EH, Newling WW, Robinson MRG, Appleyard I. The value of the measurement of serum prostate specific antigen in patients with benign prostatic hyperplasia and untreated prostate cancer. Brit J Urol 1988;62:584–589

75. Lindstedt G, Jacobsson A, Lundberg PA, Hedelin H, Pettersson S, Unsgaard B. Determination of prostate-specific antigen in serum by immunoradiometric assay. Clin Chem 1990;36:53–58
76. Palou J, Morote J. Elevated serum PSA and acute bacterial prostatitis. Urology 1990;35:373
77. Kuriyama M, Wang MC, Lee CL, Killian CS, Papsidero LD, Inaji H, Loor RM, Lin MF, Nishiura T, Slack NH, Murphy GP, Chu TM. Multiple marker evaluation in human prostate cancer with the use of tissue-specific antigen. JNCI 1982;68:99–105
78. Chu TM, Kuriyama M, Johnson B, Papsidero LD, Killian CS, Murphy GP, Wang MC. Circulating antibody to prostate antigen in patients with prostatic cancer. Transplant Proc 1984;16:481–485
79. Kuriyama M, Takeuchi T, Okano M, Fujimoto Y, Fujihiro S, Sakai S, Ban Y, Kawada Y, Nishiura T. Enzyme immunoassay for detection of serum prostate antigen and prostatic acid phosphatase-binding globulins. Jpn J Urol 1982;74:1186–1192
80. Allhoff EP, Proppe KH, Chapman CM, Lin CW, Prout GR Jr. Evaluation of prostate specific acid phosphatase and prostate specific antigen in identification of prostate cancer. J Urol 1983;129: 315–318
81. Virseda JA, Morente M, Piris MA, Melchor AM, Gil Pascual B, Gomez A. Prostate-specific antigen in the diagnosis of prostatic carcinoma. Actas Urol Esp 1985;9:89–94
82. Stromme JH, Haffner F, Johannessen NB, Talseth T, Frederchsen P, Theodorsen L. Diagnostic efficiency of biological markers in blood serum on prostate cancer: A comparison of four different markers and 12 different methods. Scand J Clin Lab Invest 1986;46:443–450
83. Siddall JK, Cooper EH, Newling DW, Robinson MR, Whelan P. An evaluation of the immunochemical measurement of prostatic acid phosphatase and prostatic specific antigen in carcinoma of the prostate. Eur Urol 1986;12:123–130
84. Wang TY, Kawaguchi TP. Preliminary evaluation of measurement of serum prostate-specific antigen level in detection of prostate cancer. Ann Clin Lab Sci 1986;16:461–466
85. Killian CS, Emrich LJ, Vargas FP, Yang N, Wang MC, Priore R, Murphy GP, Chu TM. Relative reliability of five serially measured markers for prognosis of progression in prostate cancer. JNCI 1986;76:179–185
86. Chan DW, Bruzek DJ, Oesterling JE, Rock RC, Walsh PC. Prostate-specific antigen as a marker for prostate cancer: A monoclonal and a polyclonal immunoassay compared. Clin Chem 1987;33:1916–1920
87. Guinan P, Bhatti R, Ray P. An evaluation of prostate specific antigen in prostate cancer. J Urol 1987;137: 686–689
88. Guinan P, Ray P, Bhatti R, Rubenstein M. An evaluation of the five tests to diagnose prostate cancer. Prog Clin Biol Res 1987;243A:551–558
89. Stamey TA, Kabalin JN, McNeal JE, Johnson IM, Fredha F, Redwine EA, Yang N. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. I. Untreated patients. J Urol 1989; 141:1070–1075
90. Powell CS, Fielding AM, Rosser K, Ames AC, Vaughton KC. Prostate specific antigen – A screening test for prostatic cancer? Br J Urol 1989;64:504–506
91. Brawer MK, Lange PH. Prostate-specific antigen and premalignant changes: Implications for early detection. CA 1989;39:361–375
92. Hudson MA, Bahnson RR, Catalona WJ. Clinical use of prostate specific antigen in patients with prostate cancer. J Urol 1989;142:1011–1017
93. Vessy SG, Goble M, Perro MA, Stower MJ, Hammonds JC, Smith PJ. Quantification of prostatic cancer metastatic disease using prostate-specific antigen. Urology 1990;35:483–486
94. Haapiainen PK, Permi EJ, Panniko SA, Voutilainen PE, Liewendahl K, Stenman UH, Alfthan OS. Prostate tumour markers as an aid in the staging of prostatic cancer. Br J Urol 1990;65:264–267
95. Haapiainen RK, Permi EJ, Rannikko SA, Voutilainen PE, Alfthan OS. Prostate tumour markers and differentiation grade in prostatic cancer. Br J Urol 1990;66:635–638
96. Babaian RJ. The relationship of prostate-specific antigen to digital rectal examination and transrectal ultrasonography. Findings of the American Cancer Society National Prostate Cancer Detection Project. Cancer 1992;69:1195–1200, 1992;66:635–638
97. Vessella RL, Noteboom J, Lange PH. Evaluation of the Abbott IMx automated immunoassay of prostate-specific antigen. Clin Chem 1992;38:2044–2054
98. Muchida T, Ohishi Y, Wada T, Akimoto S, Shimazaki J, Oda H, Yokoyama M, Tsukada T, Nakayama T, Akiyama A., Miki M, Meguro N, Usami M, Kotake T, Yamamoto M, Naito K. Clinical evaluation of a new kit (IMx Dainapack) for detection of serum prostate specific antigen. Acta Urol Jpn 1993;39:977–984
99. Takeuchi T, Kuriyama M, Fujihiro S, Fujimoto Y, Okano M, Nishiura T. Evaluation of serum prostate-specific antigen in urological cancers. J Surg Oncol 1983;24:157–160
100. Kuriyama M, Takeuchi T, Okano M, Deguchi T, Fujimoto Y, Fujihiro S, Ban Y, Nishiura T. A normal value of serum prostate antigen in Japanese males and its comparison with Americans. Jpn J Urol 1984;75: 802–806
101. Morishita N, Minami Y, Ogawa S, Saito Y. Purification and enzyme immunoassay of tumor markers for prostate cancer: Prostatic acid phosphatase, prostate-specific antigen and creatinine kinase BB. Jpn J Urol 1984;75:404–413
102. Hashimoto H, Yamauchi K, Tokunaka S, Yachiku S, Otsuka A, Minami S, Ishida H , Miyamoto T. The clinical evaluation of prostate-specific antigen as a serum marker of prostatic cancer. Jpn J Urol 1987;78: 253–259
103. Park YC, Kiwamoto H, Nishioka T, Tsujihashi H, Mitsubayashi S, Matsuura T, Akiyama T, Kurita T. Clinical evaluation of a prostate-specific antigen as a serum marker of prostatic cancer. Acta Urol Jpn 1987;33:883–888
104. Fuse H, Akakura K, Akimoto S, Shimazaki J. Prostatic specific antigen in serum of prostatic cancer. Acta Urol Jpn 1987;33:1049–1053
105. Yoshiki T, Okada K, Oishi K, Yoshida O. Clinical significance of tumor markers in prostatic carcinoma – comparative study of prostatic acid phosphatase, prostate specific antigen and γ-seminoprotein. Acta Urol Jpn 1987;33:2044–2049
106. Tsukamoto T, Kumamoto Y, Yamazaki K, Umehara T, Ohmura K, Miyao N. Clinical study of tumor marker

in prostatic carcinoma – clinical significance of the prostate specific antigen (PA). Jpn J Urol 1987;78: 844–852
107. Akimoto S, Akakura K, Fuse H, Shimazaki J. Prostate specific antigen in serum of the patients with prostate cancer. Acta Urol Jpn 1988;34:636–642
108. Akimoto S, Akakura K, Shimazaki J. Prostatic acid phosphatase (PAP), γ-seminoprotein (γ-Sm) and prostate specific antigen (PA) in prostatic cancer. Acta Urol Jpn 1988;34:1389–1396
109. Akimoto S, Akakura K, Shimazaki J. Comparison of 9 assay kits of prostate specific antigen in serum of prostatic cancer. Acta Urol Jpn 1988;34:2143–2148
110. Tsukamoto T, Kumamoto Y. Clinical study of tumor markers in prostatic carcinoma: An investigation on the simultaneous measurement of prostatic acid phosphatase (PAP), prostatic antigen (PA) and γ-seminoprotein (γ-Sm). Acta Urol Jpn 1988;34:987–995
111. Shinoda I, Kuriyama M, Takeuchi T, Takahashi Y, Ban Y, Kawada Y. Clinical studies on tumor markers in prostate cancer: The evaluation of PA (prostate specific antigen) and comparison with PAP and γ-Sm. Jpn J Urol 1988;79:635–642
112. Hiromoto Y, Hiramori M, Higami Y, Imamura K. The clinical evaluation of prostatic specific antigen in prostatic cancer. Jpn J Urol 1988;79:606–612
113. Yamaguchi K, Tanaka M, Kitagawa N, Kotake T, Yanagi S, Ito H. The significance of serum prostate-specific antigen, γ-seminoprotein and prostatic acid phosphatase as prostate cancer markers. Acta Urol Jpn 1989;35:987–991
114. Arai Y, Yoshiki T, Okada K, Yoshida O, Yamamoto N, Sakatoku J, Sugumura Y, Kawamura J. Clinical evaluation of serum prostatic specific antigen in prostatic cancer: Simultaneous assays of prostatic specific antigen, γ-seminoprotein and prostatic acid phosphatase in 113 newly diagnosed patients with prostatic cancer. Acta Urol Jpn 1989;35:1519–1528
115. Arai Y, Yoshiki T, Okada K, Yoshida O. Multiple marker evaluation in prostatic cancer using prostatic specific antigen, γ-seminoprotein and prostatic acid phosphatase. Urol Int 1989;44:135–140
116. Arai Y, Yoshiki T, Yamabe H, Yoshida O. Value of prostate-specific antigen measurement in predicting lymph node involvement in prostatic cancer. Urol Int 1990;45:356–360
117. Kuriyama M, Shinoda I, Kawada Y, Gohji K, Goto A, Kamidono S, Akimoto S, Shimazaki J. Studies of tumor markers in prostate cancer: A cooperative study for evaluation of Pros-Check PSA and comparison to other methods and markers. J Jpn Soc Cancer Ther 1991;26:632–643
118. Kuriyama M, Esaki N, Yamada S, Ito S, Okuno M, Takahashi Y, Shinoda I, Kawada Y, Okawa J, Miyawaki A, Kiyomi C, Todo K, Fujinaka H, Nishiyama H, Fujii A. Measurement of serum PSA values by Delfia PSA and its clinical significance on diagnosis and follow-up of prostate cancer patients. Acta Urol Jpn 1992;38: 425–431
119. Imai K, Suzuki T, Hayashi M, Shiono A, Tamura Y, Yamanaka H, Noborimaru Y. Clinical characteristics and significance of prostatic specific antigen in mass screening for prostate cancer. Kitakantoigaku 1992; 42:353–359
120. Kuriyama M, Esaki N, Shinoda I, Ito S, Yamada S, Tokuyama K, Deguchi T, Takahashi Y, Kawada Y, Akimoto S, Shimazaki J. Measurement of serum PA values by a newly developed enzyme immunoassay. Jpn J Urol 1993;84:244–250
121. Akiyama A, Yamamoto S, Hokoishi F, Ogawa M, Akizawa T, Matsumoto T, Miki M. Comparative assessment of the characteristics in various assay kit for prostate specific antigen. Jpn J Urol 1993;84:2103–2108
122. Myrtle JF. Normal levels of prostate-specific antigen (PSA). In: Catalona WJ, Coffy DS, Karr JP (eds) Clinical Aspect of Prostate Cancer. New York: Elsevier Science Publishing Co., 1989;183–189
123. Yang N. PSA values in BPH and stage A prostate cancer. In: Catalona WJ, Coffy DS, Karr JP (eds) Clinical Aspect of Prostate Cancer. New York: Elsevier Science Publishing Co., 1989;197–200
124. Kuriyama M, Yamamoto N, Shinoda I, Kawada Y, Akimoto S, Shimazaki J, Takagaki M, Ozawa M, Ohmura M. Clinical evaluation of Tandem PSA in Japanese cases and its comparison to other methods. The 81st Annual Meeting of Japanese Urological Association, Kyoto, April 1993. Jpn J Urol 1994;85:163
125. Partin AW, Carter HB, Chan DW, Epstein JI, Oesterling JE, Rock RC, Weber JP, Walsh PC. Prostate specific antigen in the staging of localized prostate cancer: Influence of tumor differentiation, tumor volume and benign hyperplasia. J Urol 1990;143:747–752
126. Lange PH, Ercole CJ, Lightner DJ, Fraley EE, Vessella R. The value of serum prostate specific antigen determinations before and after radical prostectomy. J Urol 1989;141:873–879
127. Shinoda I, Kuriyama M, Takahashi Y, Nagamai Y, Kobayashi S, Yamamoto N, Kawamoto S, Takeda A, Iwata H, Takeuchi T, Shimazu R, Tei K, Kawada Y. Clinical evaluation of a chemiluminescence immunoassay for detection of serum PSA. Urol Surg 1992;5:1059–1064
128. Weber JP, Oesterling JE, Peters CA, Partin AW, Chan DW, Walsh PC. The influence of reversible androgen deprivation on serum prostate-specific antigen levels in men with benign prostatic hyperplasia. J Urol 1989;141:987–992
129. Lee F, Torp-Pedersen ST, Caroll JT, Siders DB, Christensen-Day C, Mitchell AR. Use of transrectal ultrasound and prostate-specific antigen in diagnosis of prostatic intraepithelial neoplasia. Urology 1989;34,suppl 6:4–8
130. Hortin GL, Bahnson RR, Daft M, Chan KM, Catalona WJ, Ladenson JH. Differences in values obtained with 2 assays of prostate specific antigen. J Urol 1988;139:762–765
131. Arai Y, Yoshiki T, Oishi K, Takeuchi H, Yoshida O. The role of prostatic specific antigen in monitoring prostatic cancer and its prognostic importance. Urol Res 1990;18:331–336
132. Cooner WH, Mosley BR, Rutherfold CL Jr, Beard JH, Pond HS, Terry WJ, Igel TC, Kidd DD. Prostate cancer detection in a clinical urological practice by ultrasonography, digital rectal examination and prostate specific antigen. J Urol 1990;143:1146–1152
133. Chybowski FM, Larson Keller JJ, Bergstralh WJ, Oesterling JE. Predicting radionuclide bone scan findings in patients with newly diagnosed, untreated prostate cancer: Prostate specific antigen is superior to

all other clinical parameters. J Urol 1991;145:313–318
134. Kuriyama M, Wang MC, Lee CL, Papsidero LD, Killian CS, Inaji H, Slack NH, Nishiura T, Murphy GP, Chu TM. Use of human prostate-specific antigen in monitoring prostate cancer. Cancer Res 1981;41:3874–3878
135. Kuriyama M, Takeuchi T, Okano M, Fujimoto Y, Fujihiro S, Nishiura T, Murphy GP, Chu TM. Prostate antigen as a tumor marker of prostate cancer: (1) Changes of prostate antigen values during anti-androgen therapy. J Jpn Soc Cancer Ther 1984;19:1481–1487
136. Kuriyama M, Takeuchi T, Shinoda I, Okano M, Fujimoto Y, Fujihiro S, Nishiura T, Murphy GP, Chu TM. Prostate antigen as a tumor marker of prostate cancer: (2) Changes of prostate antigen values during anti-cancer chemotherapy. J Jpn Soc Cancer Ther 1984;19:2230–2238
137. Vernon SE, Williams WD. Pre-treatment and post-treatment evaluation of prostatic adenocarcinoma for prostate specific antigen. J Urol 1983;130:95
138. Ercole CJ, Lange PH, Mathisen M, Chiou RK, Reddy PK, Vessella RL. Prostatic specific antigen and prostatic acid phosphatase in monitoring and staging of patients with prostate cancer. J Urol 1987;138:1181–1184
139. Emtage LA, Lewis PW, Blackledge GR. The role of prostate specific antigen in the baseline assessment of patients undergoing hormone therapy for advanced prostate cancer. Br J Urol 1987;60:572–577
140. Algaba F, Chechile G, Zungri E. Correlation between tissue levels of prostatic acid phosphatase and prostate-specific antigen and hormonal response of metastatic prostatic cancer. Eur Urol 1988;14:470–473
141. Stamey TA, Kabalin JN, McNeal JE, Johnson IM, Freiha F, Redwine EA, Yang N. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. II. Radical prostatectomy treated patients. J Urol 1989;141:1076–1083
142. Stamey TA, Kabalin JN, Ferrari M. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. III. Radiation treated patients. J Urol 1989;141:1084–1087
143. Stamey TA, Kabalin JN, Ferrari M, Yang N. Prostate specific antigen in the diagnosis and treatment of adenocarcinoma of the prostate. IV. Anti-androgen treated patients. J Urol 1989;141:1088–1090
144. Kabalin JN, Hodge KK, McNeal JE, Freiha FS, Stamey TA. Identification of residual cancer in the prostate following radiation therapy: Role of transrectal ultrasound guided biopsy and prostate specific antigen. J Urol 1989;142:326–331
145. Drago JR, Badalament RA, Wiendjes MG, Smith JJ, Nesbitt JA, York JP, Neff JC. Relative value of prostate-specific antigen and prostatic acid phosphatase in diagnosis and management of adenocarcinoma of prostate. Urology 1989;34:187–192
146. Perro MA, Gillart D, Symes MO, Smith PJ. High-dose intravenous estrogen therapy in advanced prostatic carcinoma. Use of serum prostate-specific antigen to monitor response. Urology 1989;34:134–138
147. Goldrath DE, Messing EM. Prostate specific antigen: Not detectable despite tumor progression after radical prostatectomy. J Urol 1989;142:1266–1269
148. Pontes JE, Jabalameli P, Montie J, Foemmel R, Howard FD, Boyett J. Prognostic implications of disappearance rate of biologic markers following radical prostatectomy. Urology 1990;36:415–419
149. Lange PH, Lightner DJ, Medini B, Reddy PK, Vessella RL. The effect of radiation therapy after radical prostatectomy in patients with elevated prostate specific antigen levels. J Urol 1990;144:927–932
150. Kaplan I, Prestidge BR, Cox RS, Bagshaw MA. Prostate specific antigen after irradiation for prostatic carcinoma. J Urol 1990;144:1172–1175
151. Gerber GS, Chodak GW. Prostate specific antigen for assessing response to ketoconazole and prednisone in patients with hormone refractory metastatic prostate cancer. J Urol 1990;144:1177–1179
152. Lightner DJ, Lange PH, Reddy PK, Moore L. Prostate specific antigen and local recurrence after radical prostatectomy. J Urol 1990;144:921–926
153. Nativ O, Myers RP, Farrow GM, Therneau TM, Zincke H, Lieber MM. Nuclear deoxyribonucleic acid ploidy and serum prostate specific antigen in operable prostatic adenocarcinoma. J Urol 1990;144:303–306
154. Rainwater LM, Morgan WR, Klee GG, Zincke H. Prostate-specific antigen testing in untreated and treated prostatic adenocarcinoma. Mayo Clin Proc 1990;65:1118–1126
155. Carter HB, Partin AW, Epstein JI, Chan DW, Walsh PC. The relationship of prostate specific antigen levels and residual tumor volume in stage A prostate cancer. J Urol 1990;144:1167–1170
156. Morgan WR, Zincke H, Rainwater LM, Myers RP, Klee GG. Prostate specific antigen values after radical retropubic prostatectomy for adenocarcinoma of the prostate: Impact of adjuvant treatment (hormonal and radiation). J Urol 1991;145:319–323
157. Leo MB, Bilhartz DL, Bergstralh EJ, Oesterling JE. Prostate-specific antigen in hormonally treated D2 prostate cancer: Is it always an accurate indicator of disease status? J Urol 1991;145:802
158. Sceilhammer PF, El-Mahdi AM, Wright GL Jr, Kolm P, Ragle R. Prostate-specific antigen to determine progression-free survival after radiation therapy for localized carcinoma of prostate. Urology 1993;42:13–20
159. Arai Y, Yoshiki T, Yoshida O. Prognostic significance of prostate specific antigen in endocrine treatment for prostatic cancer. J Urol 1990;144:1415–1419
160. Akimoto S, Akakura K, Masai M, Isaka S, Shimazaki J. Changes in prostatic acid phosphatase, γ-seminoprotein and prostate specific antigen after endocrine therapy for stage D2 prostate cancer. Acta Urol Jpn 1990;36:783–791
161. Shinoda I. Clinical studies on tumor markers for monitoring prostate cancer patients: The evaluation of prostate-specific antigen and comparison with prostatic acid phosphatase and γ-seminoprotein. J Jpn Soc Cancer Ther 1990;2627–2639
162. Csapo Z, Brand K, Walther R, Fokas K. Comparative experimental study of serum prostate specific antigen and prostatic acid phosphatase in serially transplantable human prostatic carcinoma lines in nude mice. J Urol 1988;140:1032–1038
163. Nadji M, Tabei SZ, Castro A, Chu TM, Wang MC, Murphy GP, Morales AR. Prostatic specific antigen: An immunohistologic marker for prostatic neoplasms. Cancer 1981;48:1229–1232
164. Okano M, Deguchi T, Takeuchi T, Kuriyama M, Nishiura T. An immunohistochemical study of pros-

tate antigen using an enzyme-labeled antibody technique. Acta Urol Jpn 1983;29:1369–1371
165. Stein BS, Vangore S, Petersen RO, Kendall AR. Immunoperoxidase localization of prostate-specific antigen. Am J Surg Pathol 1982;6:553–557
166. Epstein JI, Eggleston JC. Immunohistochemical localization of prostate-specific acid phosphatase and prostate-specific antigen in stage A2 adenocarcinoma of the prostate: Prognostic implications. Hum Pathol 1984;15:853–859
167. Ghazizadeh M, Kagawa S, Maebayashi K, Izumi K, Kurokawa K. Prostatic origin of metastases: Immunoperoxidase localization of prostate-specific antigen. Urol Int 1984;39:9–12
168. Purnell DM, Heatfield BM, Trump BF. Immunocytochemical evaluation of human prostatic carcinomas for carcinoembryonic antigen, nonspecific cross-reaction antigen, beta-chorionic gonadotrophin, and prostate-specific antigen. Cancer Res 1984;44: 285–292
169. Ford TF, Butcher DN, Masters JR, Parkinson MC. Immunocytochemical localization of prostate-specific antigen: Specificity and application to clinical practice. Br J Urol 1985;57:50–55
170. Tell DT, Khoury JM, Taylor HG, Veasey SP. Atypical metastasis from prostate cancer. Clinical utility of the immunoperoxidase technique for prostate-specific antigen. JAMA 1985;253:3574–3575
171. Deguchi T, Doi T, Ehara H, Ito S, Takahashi Y, Nishino Y, Fujihiro S, Kawamura T, Komeda H, Horie M, Kaji H, Shimokawa K, Tanaka T, Kawada Y. Detection of micrometastatic prostate cancer cell in lymph node by reverse transcriptase-polymerase chain reaction. Cancer Res 1993;53:5350–5354
172. Keilor JS, Ateman K. The response of poorly differentiated prostatic tumors to staining for prostate specific antigen and prostatic acid phosphatase: A comparative study. J Urol 1987;137:894–896
173. Kamoshida S, Tsutsumi Y. Extraprostatic localization of prostatic acid phosphatase and prostate-specific antigen: Distribution in cloacogenic glandular epithelium and sex-dependent expression in human anal gland. Hum Pathol 1990;21:1108–1111
174. Okano M. Localization of prostate specific antigen and its clinical significance. Jpn J Urol 1990;81:847–854
175. Babaian RJ, Miyashita H, Evans RB, Ramirez EI. The distribution of prostate specific antigen in men without clinical or pathological evidence of prostate cancer: Relationship to gland volume and age. J Urol 1992;147:837–840
176. Labric F, Dupont A, Suburu R, Cusan L, Tremblay M, Gomez J-L, Emond J. Serum prostate specific antigen as pre-screening test for prostate cancer. J Urol 1992;147:846–852
177. Dalkin BL, Ahmann FR, Kopp JB. Prostate specific antigen levels in men older than 50 years without clinical evidence of prostatic carcinoma. J Urol 1993;150:1837–1839
178. Kane RA, Littrup PJ, Babaian RJ, Grago JR, Lee F, Chesley A. Prostate-specific antigen levels in 1695 men without evidence of prostate cancer. Findings of the American Cancer Society National Prostate Cancer Detection Project. Cancer 1992;69:1201–1207
179. Babaian RJ, Fritsche HA, Evans RB. Prostate-specific antigen and prostate gland volume: Correlation and clinical application. J Clin Lab Anal 1990;4:135–136
180. Benson MC, Whang IS, Pantuck AP, Ring K, Kaplam SA, Olsson CA, Cooner WH. Prostate specific antigen density: A means of distinguishing benign prostatic hypertrophy and prostate cancer. J Urol 1992; 147:815–816
181. Benson MC, Whang IS, Olsson CA, McMahon DJ, Cooner WH. The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specific antigen. J Urol 1992;147:817–821
182. Lee F, Littrup PJ, Loft-Christensen L, Kelly BS, McHugh TA, Siders DB, Michell AB, Newly JH. Predicted prostate specific antigen results using transrectal ultrasound gland volume. Differentiation of benign prostatic hyperplasia and prostate cancer. Cancer 1992;70:211–220
183. Watt KWK, Lee P-J, M'Timkulu T, Chan W-P, Loor R. Human prostate-specific antigen: Structural and functional similarity with serine proteases. Proc Natl Acad Sci, USA 1986;83:3166–3170
184. Stenman U-H, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and α-1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:322–226
185. Lilja H, Christensson A, Dahlen U, Matikainen M-T, Nilsson O, Pettersson K, Lovgren T. Prostate-specific antigen in serum occurs predominantly in complex with α-1-antichymotrypsin. Clin Chem 1991;37: 1618–1625
186. Wood WG, Sloot van der E, Bohle A. The establishment and evaluation of luminescent-labelled immunometric assay for prostate-specific antigen – α-1-antichymotrypsin complexes in serum. Eur J Clin Chem Clin Biochem 1991;29:787–794
187. Lilja H, Cockett AT, Abrahamsson PA. Prostate specific antigen predominantly forms a complex with α-1-antichymotrypsin in blood. Implication for procedures to measure prostate specific antigen in serum. Cancer 1992;70:230–234
188. Kuriyama M, Ueno K, Kawada Y. Clinical significance of PSA and PSA-ACT complex for diagnosis and follow up of prostate cancer patients. Proc The 4th Prostate Cancer Workshop, Tokyo, 1993
189. Christensson A, Bjork T, Nilsson O, Dahlen U, Matikainenn M-T, Cockett ATK, Abrahamsson P-A, Lilja H. Serum prostate-specific antigen complexed to α-1-antichymotrypsin as an indicator of prostate cancer. J Urol 1993;150:100–105
190. Demura T, Watarai Y, Togashi M, Hirano T, Ohashi N, Koyanagi T. Measurement of prostate specific antigen and γ-seminoprotein ratio: A new means of distinguishing benign prostatic hyperplasia and prostate cancer. J Urol 1993;150:1740–1745
191. Graves HCB, Wehner N, Sramey TA. Comparison of a polyclonal and monoclonal immunoassay for PSA: Need for an international antigen standard. J Urol 1990;1516–1522
192. Kuriyama M, Akimoto S, Akaza H, Arai Y, Usami M, Imai K, Tanaka Y, Yamazaki H, Kawada Y, Koiso K, Yoshida O, Kotake T, Yamanaka H, Machida T, Aso Y, Shimazaki J. Comparison of various assay system for prostate-specific antigen standardization. Jpn J Clin Oncol 1992;22:393–399